PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jaakko PERE, et al.

Docket No: Q95394

Appln. No.: 10/582,525

Group Art Unit: 1791

Confirmation No.: 1046

Examiner: Anthony J. CALANDRA

Filed: January 19, 2007

For: PROCESS FOR PREPARING MECHANICAL PULP

SUBSTITUTE SPECIFICATION - CLEAN

(18 PAGES)

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE
23373
CUSTOMER NUMBER

Date: April 5, 2010

Process for preparing mechanical pulp

The present invention relates to a process in accordance with the preamble of Claim 1 for preparing mechanical pulp. The invention also relates to a process for decreasing the energy consumption of mechanical pulping processes based on refining chips, according to the preamble of Claim 17.

Chemical and mechanical pulps possess different chemical and fibre-technical properties and thus their use in different paper grades can be chosen according to these properties. Many paper grades contain both types of pulps in different proportions according to the desired properties of the final products. Mechanical pulp is used, when necessary, to improve or to increase the stiffness, bulkiness or optical properties of the product.

In paper manufacture, the wood material must first be defibred. Mechanical pulp is mainly manufactured by means of grinding and refining methods, in which the raw wood material is subjected to periodical pressure impulses. Due to friction heat, the structure of the wood is softened and its structure loosened, finally leading to the separation of the fibres from one another (Virkola, 1983). However, only a small part of the energy brought into the system is used for separating the fibres; the major part being converted into heat. Therefore, the total energy economy of the defibration is very poor.

Several methods for improving the energy economy of mechanical pulping are suggested in prior art. Some of these are based on the pre-treatment of chips by, e.g., water or acid (FI Patent Specifications Nos. 74493 and 87371). Methods are also known, which comprise treating the raw material with enzymes to reduce the consumption of pulping energy. Thus, the Finnish Patent Application No. 895676 describes an experiment in which once-refined pulp was treated with a xylanase enzyme preparation. It is stated in the application that this enzymatic treatment would, to some extent, decrease the energy consumption of pulping. In the said prior art publication, the possibility of using cellulases is also mentioned but no examples of these are given nor are their effects shown. As far as isolated enzymes are concerned, in addition to hemicellulases, the interest has been focused on lignin modifying enzymes, such as laccase enzyme (Jokinen and Savolainen, 1991). A treatment using the laccase enzyme did not, however, have an effect on the energy consumption (Jokinen and Savolainen, 1991).

The Patent Specification EP 0429 422 suggests the use of laccase treatments in mechanical pulping between the first and the second refining treatments. This specification states that the laccase treatment decreases the energy consumption of the

refining process. The Patent Specification WO 93/23606, in turn, suggests a treatment with phenol oxidase enzymes after the last refining or grinding treatments. The said treatment had no effect on the energy consumption of the refining treatment but it has been said to have an influence on the strength of paper or board.

As the energy in defibration is mainly absorbed by the amorphous part of the paper furnish only, i.e., hemicellulose and lignin, an increase in the amorphousness of the raw material improves the energy economy of the defibration. The Patent Specifications WO 94/20666 and WO 94/20667 suggest that the amorphousness of the raw material be increased in connection with mechanical pulping by treating the raw material with a suitable enzyme that reacts with the crystalline, insoluble cellulose of the raw material. The Patent Specification WO 94/20666 suggests that the raw material be treated with an enzyme preparation, the main cellulase activity of which consists of cellobiohydrolase activity. The Patent Specification WO 94/20667 suggests that an enzyme preparation be used for the same purpose, containing cellobiohydrolase activity and mannanase activity. The examples of the said specifications deal with rough wood, such as the long-fibre fraction of fractioned TMP spruce pulp, once-refined TMP spruce pulps (with freeness values of CSF 450 – 550) or TMP pulps refined to different freeness levels (30 – 300). If a synergistically acting cellulase enzyme product, i.e., cellulase, was used in connection with the manufacture of mechanical pulp, containing both cellobiohydrolase and endoglucanase, the treatment resulted in the hydrolysis of the insoluble cellulose and, thus, in the weakening of the strength properties of the pulp.

The Patent Specification US 6,267,841 describes a manufacturing method of thermo-mechanical pulp, wherein the pulp is treated with enzyme between the first and the second refining processes. It also suggests the treatment of chips with enzymes before the first refining. The specification cites enzymes, such as pectinase, xylanase, laccase, cellulase or the mixtures thereof. The specification gives no numerical values of any energy savings obtained.

In addition to the afore-mentioned isolated enzymes, the application of growing white rot fungi in the manufacture of mechanical pulp has also been studied. Carried out before defibration, such a treatment with a white rot fungus has been found to decrease the specific energy consumption and to improve the strength properties of these pulps (Setliff et al., 1990, Leatham et al., 1990 and Akhtar et al., 1992). The drawbacks of these treatments with the white rot fungus are, however, the long treatment times needed (mostly weeks); the decreased yield (85 to 95 %), the difficulty to control the process and the impaired optical properties.

Generally, the different enzymatic treatments according to prior art have been applied to raw wood material, which has been defibred to a certain extent already during the manufacturing process. According to a general view, the enzymatic treatment is not as effective when applied to chips directly, because it is difficult to make the enzyme preparation to be effectively absorbed into the fibres of a raw material that is in the form of chips. In a native form of chips, the surface area of the raw wood material is not sufficient for an effective enzymatic treatment to take place. Another reason is that a major part of the capillaries of the wood are too small to receive any enzyme molecules (Grethlein, H.E. Biotechnology, February 1985, pp. 155 to 160).

According to prior art, the pulping liquor is made to penetrate the chips used in pulp cooking by treating the chips with pressure shocks in the presence of the pulping liquor. In the Vilamo method, for example, the chips are treated in the presence of the cooking liquor by varying the pressure from a pressure of 4.5 kp/cm^2 and a treatment time of 10 – 16s to a pressure of 2 kp/cm^2 and a treatment time of 5 – 6s, the treatment being repeated 6 – 8 times at 1-minute intervals /Rydholm, 1965).

The Patent Specification WO 95/09267 suggests treating the chips, which are used in pulp cooking, with a chemical solution by subjecting the chips to a vacuum and making the chemical solution penetrate the wood fibres by means of a pressure shock. According to the application, the chemical solution can be cooking liquor that contains, for example, catalysts and enzymes. The object of the invention is, thus, to be able to decrease the amount of lignin in order to diminish the need of decreasing the residue lignin at the final stage of cooking. However, the application does not describe in detail, whether or not the enzymes penetrate the wood cells successfully and whether or not the enzymes have any effect on the decrease of the amount of lignin.

The US Patent 5,374,555 suggests the removal of lignin from the lignocellulose material by means of a protease enzyme. To enhance the enzymatic treatment, the patent suggests a mechanical treatment of chips, for example, in a screw clamp. The patent specification reminds that cellulase can be used as a pre-treatment enzyme for the chips or the pulp, but it does not suggest carrying out a treatment with cellulase in connection with the mechanical processes. The purpose of the patent is not to save energy but to remove lignin, and there are no observations relating to energy economy. While the application suggests a protease treatment of the wood material, which is used both in the manufacture of mechanical pulp and in that of chemical pulp, the main issue is the removal of lignin as a pre-treatment in the manufacture of chemical pulp.

The Patent Specification WO 97/40194 suggests changing the structure or the composition of the wood by adding to the compressed chips fungal or bacterial cultures or products, such as enzymes obtained from them, by means of pressure. The purpose of the compression is to make cracks and fractures in the wood. When the chips are released from the compression, microbes of their products, while the chips expand, are absorbed by the structures of the wood partially by the virtue of under pressure, partially by the capillary action. The application suggests, among others, fungi from the genera *Ceriposiophsis*, *Phanerochaete* and *Ophiostoma*. Regarding enzymes, lipolytic, proteolytic, linginolytic, cellulolytic and hemicellulolytic enzymes are mentioned. The patent specification describes the absorption of the enzyme preparation Clariant Cartazyme HS™ (xylanase) into the compressed chips after releasing the pressure. Liquid was removed after the treatment, and mechanical pulp was prepared from the chips. In that case, the amount of energy consumed was 7.5% less than in the case of chips that were treated with a buffer only. In another test, the enzyme preparations Clariant Cartazyme NS™ (xylanase) and Sigma porcine pancreas Lipase L-3126 were treated. In that case, the amount of energy consumed was 12.5% less than when treated with a buffer only. The specification mentions no preservation of the optical properties of the pulp. According to the specification, the highest energy savings were made by combining enzyme preparations originating in different sources, of which the amount of a lipase of a mammalian origin, in particular, was considerable. The amount of the other enzymes used was also fairly high, which makes one suspect that the energy savings achieved were not particularly cost-effective.

Eriksson and Heitman (1998) describe tests, wherein pieces of wood (with a size of 1x1x1.5 inches) were treated with a cellulase enzyme mixture, after which the pieces were ground and the energy consumed by the grinding was studied. The absorption of the enzyme mixture into the pieces of wood was facilitated by subjecting the pieces to a vacuum. The treatment was not observed to have any effect on the consumption of the grinding energy.

One problem with the manufacturing methods of mechanical pulp according to prior art is their great energy consumption. While attempts have been made to improve the beating degree and the energy economy with the aid of enzymatic treatments, hardly any energy savings have been made and, often, they have resulted in the weakening of the strength properties of the pulp (cellulase treatment) or in the darkening of the pulp and an impairment in the optical properties (laccase treatment). Furthermore, it has not always been possible to make the enzyme solution effectively act on the wood. In some cases,

the preparation of the enzyme composition used in the tests may have included time-consuming stages and/or been otherwise uneconomic.

The purpose of the present invention is to remove at least some disadvantages of prior art and to provide an improved method for the manufacture of mechanical pulp. To be more precise, the object of the invention is to provide a pre-treatment method of chips to be used before preparing the mechanical pulp.

In connection with the present invention, it has surprisingly been observed that chips can be pre-treated with an enzyme preparation that has synergistically acting enzyme activities. In that case, the enzyme preparation does not need to contain any certain isolated enzyme activity only, but an enzyme preparation containing different enzyme activities can be used directly as the enzyme preparation.

The treatment according to the method can be applied to the chips directly. As the enzymatic treatment takes place at an early stage of the pulping process, savings in the refining energy are then as high as possible.

According to the method of the present invention, the chips are pre-treated with an enzyme that is capable of degrading the structural parts of the wood, after which mechanical pulp is manufactured from the chips by refining. It is preferable to carry out the enzymatic treatment by compressing the chips and by bringing the compressed chips in a liquid phase into contact with the enzyme composition to absorb the enzyme composition into the chips. The enzyme composition preferably contains both cellobiohydrolase and endoglucanase. It is particularly preferable for the composition to contain an effective amount of both cellobiohydrolase and endoglucanase. Enzyme preparations containing cellobiohydrolase and endoglucanase in a ratio of 20:1 – 1:20, indicated as the weight ratio of the proteins, are preferable.

According to some preferable embodiments of the invention, the amount of endoglucanase compared with that of cellobiohydrolase is higher than what is naturally produced by the industrial production strains of cellulase, such as *Trichoderma*, in their growth media.

To be more precise, the method according to the invention is mainly characterized in that which is presented in the characterizing part of Claim 1.

The method according to the invention is also characterized in that which is presented in the characterizing part of Claim 17.

The invention provides several considerable advantages. When using the methods according to the preferable embodiments of the invention, considerably lower amounts of energy are consumed than in the methods according to prior art. The energy saving can be as much as 20% compared with a method, wherein the chips are not treated with the enzyme preparation.

When treating the chips by the methods according to the preferable embodiments of the invention, the strength of the pulp was not weakened; on the contrary, it improved to some extent. The optical properties also remained good. Thus, when treating the chips with the methods according to the preferred embodiments of the invention, it was possible to improve the quality of the pulp.

According to prior art, treating the raw material by means of a non-optimized cellulase enzyme product resulted in the hydrolysis of the insoluble cellulase and, thus, in the weakening of the strength properties of the pulp. In connection with the present invention, it was surprisingly observed that the enzyme preparation containing cellobiohydrolase and endoglucanase did not necessarily result in a loss of the pulp strength.

According to the preferred embodiments of the invention, the enzyme preparation is produced in a host organism, which excretes the enzyme preparation out of the cell, whereby the enzyme preparation does not need to be isolated from the cell. It is also especially advantageous to use a genetically modified organism as the production host, producing the desired enzyme preparation directly in the growth medium. This provides the considerable advantage that the used enzyme activity does not need to be isolated from the host organism or its growth medium but, for example, the growth medium of the host organism can be directly used.

In the following, the invention is described with the aid of a detailed description and some examples, the purpose of which, however, is not to limit the scope of the invention.

The enzymes that participate in the modification and degradation of cellulose are commonly called "cellulases". These enzymes include endo-β-glucanases, cellobiohydrolases and β-glucosidase. Countless organisms, such as various wood rotting fungi, moulds and anaerobic bacteria are able to produce some or all of these enzymes. Depending on the type of organism and cultivation conditions, these enzymes are produced extracellularly in various ratios and amounts.

The term "enzyme preparation" used in this application refers to any product that contains at least one enzyme or a structural part of the enzyme. Accordingly, the enzyme

preparation can be, for example, a growth medium containing the enzyme(s), an isolated enzyme or a mixture of two or more enzymes. "Cellulase" or "cellulase enzyme preparation", in turn, refers to an enzyme preparation containing at least one of the above-mentioned cellulase enzymes. The "enzyme composition" in this application means the same as the enzyme preparation. In addition to the enzymes, the enzyme preparation or the enzyme composition may also contain, for example, buffers, stabilizers, preservatives or other necessary additives.

The "cellobiohydrolase activity" in this application refers to an activity that is capable of modifying the crystalline parts of the cellulose. The cellobiohydrolase I and II activities refer to the main activities of the cellobiohydrolase produced by *Trichoderma* or to the corresponding activities produced by some other organism. The endoglucanase activity in this application refers to an activity capable of modifying the amorphous parts of the cellulose. The endoglucanase I and the endoglucanase II activities refer to the main activities of the endoglucanase produced by *Trichoderma* or to the corresponding activities produced by another organism.

An enzyme preparation containing "an effective amount" of cellobiohydrolase and endoglucanase refers to an enzyme preparation, in which the effect of each enzyme on the chips can be measured as a reduction in the energy consumption of the refining. The effective amount of cellobiohydrolase and endoglucanase provides a decrease of at least 3%, preferably at least 5%, more preferably at least 8%, most preferably at least 10% in the energy consumption of the refining.

When so desired, the methods according to the invention can be combined with treatments carried out with other enzymes, such as hemicellulases (e.g., xylanases, glucuronidases and mannanases) or esterases. In addition to these enzymes, additional enzyme preparations containing β-glucosidase activity can be used in the present processes, because this kind of β-glucosidase activity prevents the end product inhibition caused by cellobiose.

Cellobiohydrolase and endoglucanase enzyme preparations are produced by growing suitable micro-organism strains, known to produce cellulase. The strains are preferably production strains that are used industrially. The growth medium used can be, for example, a simple cellulosic substrate (1% Solka floc), which the necessary trace elements have been added to (Mandels and Weber, 1969). The production strains can be bacteria, fungi or moulds. As examples, the micro-organisms belonging to the following families can be mentioned:

Trichoderma (e.g. *T. reesei*), *Aspergillus* (e.g. *A. niger*), *Phanerochaete* (e.g. *P. chrysosporium*; Covert et al., 1992), *Penicillium* (e.g. *P. janthinellum*, *P. digitatum*), *Streptomyces* (e.g. *S. olivochromogenes*, *S. flavogriseus*), *Humicola* (e.g. *H. insolens*), and *Bacillus* (e.g. *B. subtilis*, *B. circulans*, Ito et al., 1989). Other white rot fungi can also be used, strains belonging to families, such as *Phlebia*, *Ceriporiopsis* and *Trametes*.

It is also possible to produce cellobiohydrolases, endoglucanases or their structural parts by means of strains, which have been genetically improved to produce specifically these proteins, or by other genetically modified production strains, to which genes coding for these proteins have been transferred. When the genes of the desired protein have been cloned (Teeri et al., 1983), it is possible to produce the protein or its part in a desired host organism. The desired host may be the *Trichoderma* mould (EP 244 234, Mitsuishi et al., 1990), yeast (Penttilä et al., 1988), some other mould, from families such as *Aspergillus* (van den Hondel et al., 1992), a bacterium or any other micro-organism, whose genetics are sufficiently well-known.

According to the preferred embodiments of the invention, the desired cellobiohydrolase and endoglucanase are produced by means of the mould strain *Trichoderma*, preferably the strain *T. reesei*.

The said strain is a generally used production organism and its cellulases are fairly well known. *T. reesei* synthesizes two cellobiohydrolases, which are later referred to as CBH I and CBH II, several endoglucanases, of which EGI and EGII are the main activities, and at least two β-glucosidases (Chen et al, 1992). The biochemical properties of these enzymes on pure cellulosic substrates have been extensively described. Endoglucanases are typically active on soluble and amorphous substrates (CMC, HEC, β-glucan), whereas the cellobiohydrolases are able to hydrolyze crystalline cellulose. The cellobiohydrolases act clearly synergistically on crystalline cellulose, but their hydrolysis mechanisms are supposed to be different from each other. The present knowledge of the hydrolysis mechanisms of cellulases is based on results obtained on pure cellulase preparations, and may not be valid in cases, where the substrate also contains other components, such as lignin or hemicellulose.

The cellulases of *T. reesei* (cellobiohydrolases and endoglucanases) do not essentially differ from each other with respect to their optimal external conditions, such as pH or temperature. Instead, they differ from each other with respect to their ability to hydrolyze and modify cellulose in the raw wood material.

As far as their activities are concerned, the cellobiohydrolases I and II also differ to some extent from each other, and so do the endoglucanases I and II. In the preferred embodiments of this invention, however, it seems that the ratio of the cellobiohydrolases to the endoglucanases is more important than the interrelation between the various cellobiohydrolases or the various endoglucanases.

Trichoderma reesei naturally produces various cellulase components in its growth medium, the amount and the interrelation of them depending on the production strain and the external conditions used. For the wild type of *Trichoderma reesei*, the following relative amounts of the main cellulases have been proposed: CBH I 60%, CBH II 20%, EG I 10% and EG II 10% (Ståhlberg, 1991). In that case, the ratio of the cellobiohydrolases to the endoglucanases is about 4:1.

In this invention, it was observed that the preferred enzyme mixtures for the method according to the invention included those containing both cellobiohydrolase enzymes and endoglucanase enzymes. While not wanting to commit ourselves to any theories, it very strongly seems that the method according to the invention needs both cellobiohydrolase enzymes and endoglucanase enzymes, because the endoglucanase is capable of preparing, in the chips, objects that the cellobiohydrolase is able to act on. As neither activity alone is able to provide the desired effect, the cellobiohydrolases and the endoglucanases must work in synergy. According to the preferred embodiments of the invention, the ratio of the cellobiohydrolases to the endoglucanases, indicated as the weight ratio of the proteins, is preferably 20:1 – 1:20, more preferably 9:1 – 1:9, more preferably 5:1 – 1:5, and more preferably 3:1 – 1:3, most preferably 2:1 – 1:2, and even more preferably about 1:1. Accordingly, the most preferable cellulase compositions are those, wherein the weight ratio of the cellobiohydrolases and the endoglucanases is close to 1:1. However, an energy saving effect can even be provided by a weight ratio deviating from this, if the endoglucanase used has a very strong activity so that even a small amount is sufficient to provide the desired effect.

The preferred embodiments of the invention also include an enzyme preparation, wherein the portion of endoglucanases in the preparation is 2 – 60% by weight. Even more preferred is a preparation, wherein the portion of endoglucanases in the preparation is 20 – 55% by weight and the most preferred is one, wherein the portion of endoglucanases is 45 – 50% by weight. Such an amount of endoglucanases can be reached by increasing the amount of either EGI or EGII, or both, in the preparation. If the amount of EGI is increased exclusively, the amount of EGI in the preparation should reach a level of 15 – 45% by weight. This is also true, if only the amount of EGII is increased.

US Patent No. 5,874,293, for example, describes an enzyme preparation that is produced by the strain *Trichoderma* (ALKO 3529) that overproduces EGII. The ratio of CBH:EG in the growth medium produced by the strain is estimated to be 1 – 1.4:1. It would be advantageous to use the growth medium produced by such a strain, for example, in the present invention. The publication Karhunen et al. (1993) describes a *Trichoderma* host that is modified to overproduce the EGI enzyme. The growth medium of this host could also be used in the present invention. Generally, preferable enzyme mixtures according to the preferred embodiments of this invention include those, wherein the amount of endoglucanase is higher than what the cellulase-producing micro-organisms, such as *Trichoderma*, especially *T. reesei*, would naturally produce in their growth media.

The modified cellulase preparation herein refers to a preparation, wherein the ratio of CBH and EG components has been changed by methods that are well-known to average experts. Such methods include, e.g., the genetic modification of a host organism so that the host organism produces a novel cellulase compound in its growth medium. Other viable methods of manufacturing modified cellulase preparations include the fractioning of a cellulase-containing growth medium or combining different cellulase mixtures.

The host organism can be modified genetically to produce the desired cellobiohydrolases and endoglucanases in a desired proportion. For example, the genetic modification of the strains of the family *Trichoderma* can be carried out by the methods described in the patent EP 244234 or in the publication Suominen et al., 1993. Preferable enzyme preparations to be used in the embodiments of this invention include those, wherein the mould *T. reesei* is modified to overproduce EG I and/or EG II enzymes. The overproduction host may also have been modified so as to produce less of some cellobiohydrolase activities, especially the CBH I or CBH II activities, if any, or to produce less endoglucanases, if any, especially the EG I and/or EG II activities. However, it should be noticed that in the enzyme preparations according to the preferred embodiments of the invention, there should be cellobiohydrolase activities; therefore, adding endoglucanase activities to the enzyme preparations is more advantageous than decreasing cellobiohydrolase activities or removing the endoglucanases.

Corresponding enzyme preparations can also be manufactured by purifying suitable cellobiohydrolase and endoglucanase enzymes and combining the same in advantageous proportions, or by adding to an enzyme preparation, which is produced by a non-modified host, the desired enzyme activities, for example, the EGI and EGII activities.

The strains, which are capable of overproducing EGI and EGII enzymes, can be constructed, for example, by transferring genes coding for these enzymes (*egl1* Penttilä et al. 1986 and *egl2* Saloheimo et al. 1988) to a selected *Trichoderma* host as several copies or to replace some genes of *Trichoderma*, such as the *cbh1* and *cbh2* genes that code for cellobiohydrolases, as described in the publication Suominen et al. (1993). The said genes can be expressed under a strong *cbh1* promoter, as described in the publication Paloheimo et al. (1993).

When manufacturing genetically modified hosts, the *T. reesei* strain QM6a, for example, can be used as a host, especially the strains QM9414 and Rut C – 30, which are developed from the same for the production of cellulase, or strains developed from them, which produce less protease.

According to the preferred embodiments of the present invention, the enzyme preparation is manufactured by means of a host organism, which is modified to produce cellobiohydrolases and endoglucanases in a desired proportion in its growth medium. Alternatively, endoglucanase I and/or II enzymes are added to a growth medium, which is produced by a non-modified host organism that naturally produces cellulases in its growth medium, the enzymes having either been produced by a micro-organism that is modified to overproduce these enzymes, or isolated and possibly purified from the growth medium. In the manufacture of the enzyme preparation, the above-mentioned methods can also be combined. The cellobiohydrolase and the endoglucanase can be separated from the growth medium of the production host by means of several known methods. In these methods of separation, typically, various purifying techniques are combined, such as precipitation, ion exchange chromatographic and affinity chromatographic as well as gel chromatographic methods.

The enzyme preparations can be manufactured by means of the mould *Trichoderma* or some other production host. Genes that code for cellobiohydrolase and endoglucanase can originate in *Trichoderma* or some other host that produces the preferable cellobiohydrolase and endoglucanase activities; and the said activities in the enzyme preparation can be from the same or a different origin.

The treatment according to the present invention is applied to chips. The raw wood material is chipped in a normal manner so that the chip length is about 15 – 25 mm. Before the treatment, the chips can be graded by removing oversize and too thick chips and fines.

In the method according to the invention, the chip material is typically compressed by at least 10%, generally 10 – 30% of its original bulk volume. The chips are compresses in a ratio of 1:2 – 1:10. A ratio of compression of at least 1:4 is preferably used. The compression treatment is preferably carried out by a method, wherein the chips are compressed without a considerable circular motion, because the object is not to crush the pieces of chips but make microscopic cracks in the raw wood material. In terms of technicality, the compression can be implemented by various means, e.g., in a screw clamp or by a hydraulic press. In the compression treatment, the impregnated chips are treated for a sufficient time in conditions favourable for the activity of the enzyme, after which the chips are processed in a normal manner before refining, including pre-heating with steam before feeding them into the refiner.

The method according to the invention is not limited to a certain raw wood material but can generally be applied to both softwood and hardwood, such as the species of the *Pinaceae* order (e.g., the *Picea* and *Pinus* families), the species of the *Salicaceae* order (e.g., the *Populus* family) and the species in the *Betula* family.

The compressed chips are brought into contact with the enzyme preparation in a liquid phase. This is best carried out so that the chips are compressed in an enzyme solution. The proportion of the liquid and the chips is preferably selected so that the liquid is able to effectively act on the chips. Thus, the proportion of liquid to the chips can be 10:1 – 2:1, and it is preferably 6 – 8:1. The compression pressure can be 10 – 20 MPa, and it is preferably 12 – 15 MPa. The duration of the compression/absorption stage should be at least 1 min; the duration is preferably 5 – 100 min and generally 10 – 30 min. After releasing the compression, the chips are allowed to return to their original volume under the enzyme solution, whereby the enzyme solution is impregnated into the chips.

At the compression/absorption stage, the pH and the temperature of the enzyme solution should be suitable for the functioning of the enzyme preparation. For the cellobiohydrolases and the endoglucanases, the pH should preferably be within a range of pH 3 – 10, preferably pH 4 – 8, and the temperature should be 20 - 55°C, preferably 30 - 45°C. In order for the enzyme to act on the chips before refining, the chips are treated for a sufficiently long time in the conditions mentioned above. The treatment time greatly varies depending on the properties (size, thickness) of the chips, sort of wood, compression treatment, enzyme preparation, operational conditions etc., and a suitable treatment time must be specified for each case separately. In terms of costs, as short a time as possible is advantageous but in terms of process technology, there are no

obstacles for a treatment of several hours. Typically, the treatment time can be within a range of 1 – 24h, preferably 1 – 12h.

The amount of enzyme preparation used in the invention in the treatment of chips is selected so that the amount of free sugars released in the solution is preferably about 0.1 – 1.0% of the original dry matter. A suitable dosage, determined as total protein, is 0.1 – 7mg of protein per g of chips, preferably 3 – 6mg of protein per g of chips (as dry matter).

In the present invention, mechanical pulp is manufactured by refining chips that are treated with an enzyme to obtain a drainability value, which is preferably at least 100ml CSF, more preferably 40 – 80ml CSF. Surprisingly, it was observed that the method according to the invention yielded energy savings of 13%, preferably 15% and most preferably as much as 20%.

It seems that the enzymatic treatment according to the present invention is advantageous, when combined with the manufacture of mechanical pulp by the refining method, in particular, and when refining the pulp into drainability of 100 CSF or lower.

The invention provides considerable advantages. Accordingly, it can be used to considerably reduce the specific energy consumption of refining; in accordance with the preferred embodiments of the invention, as much as 20% lower energy consumption can be achieved than with untreated source materials, as the examples below indicate. By means of a suitable enzyme preparation, the properties of the mass can also be improved. Using the solutions according to the preferred embodiments of the invention, a high yield is obtained in the manufacture of mechanical pulp by refining, the quality of the pulp is good, the strengths are maintained, the optical properties are good, and the method is easy to connect to the present processes.

The invention can be applied to all manufacturing methods of mechanical pulp, such as the manufacture of thermo-mechanical pulp (TMP) and refined mechanical pulp (RMP).

In the following, the invention is described in detail with the aid of a few examples of application.

Example 1

Enzymatic treatment of chips

Enzymatic treatments with a cellulase mixture were carried out on sorted spruce sapwood chips (ø 7mm), using an enzyme dosage of 6.3mg of protein per g of chips (as dry matter), a commercial enzyme preparation produced by the *Trichoderma* strain, wherein

the weight proportions of CBH:EG were defined as 1:1. To enhance the enzymatic treatment, a compression treatment was exerted on the chips using a PREX hydraulic press. In the hydraulic compression, a chip lot (200g) was compressed in the enzyme solution into a volume that was about 20% smaller than the original, using a compression load of 48t (14 Mpa). The ratio of liquid to wood was 11:4 and the duration of the compression/absorption stage was 10min. After releasing the compression, the chips were allowed to return to their original volume under the enzyme solution, whereby the enzyme solution was impregnated into the chips. As a reference, an otherwise similar treatment was used, but without the enzyme. In the compression treatment, neither visual nor microscopic changes were perceived in the chips. After the compression treatment, the chips (+ the compression solution) were transferred into a rotary air oven for further processing. The treatment was carried out in atmospheric pressure and at a temperature of 45°C. The amount of carbohydrates released in the treatment solution, as reducing sugars, was defined after 6 and 22 h. The result obtained was compared with a treatment, wherein the compression treatment of chips was omitted. The results are shown in Table 1.

Table 1. The amount of carbohydrates released in the solution (after 6 and 22h) from spruce sapwood chips in the enzymatic treatment. The amount of dissolved carbohydrates is calculated as per cent of the original dry matter.

Treatment	*Dissolved carbohydrates, %, dry matter*	
	6h	22h
Compression treatment	0.71	1.06
No compression treatment	0.04	0.26

On the basis of the results, it was stated that the impregnation of the enzyme provided by means of the compression treatment considerably enhanced the release of soluble carbohydrates from the chips compared with a case, wherein no compression treatment was carried out.

Example 2

Effect of the enzymatic treatment on the beatability of chips

The effects of the combined compression/absorption and enzymatic treatments on the beatability of the chips were examined by means of a blade refiner. The equipment used in the tests contained the actual refiner and an accurate energy measuring system connected thereto. The refiner chamber of the blade refiner consisted of a cylinder provided with counter blades (20 in number) and a rotary rotor having four wing-like blades. Several refining operations (125g dry matter per refining) were carried out for each specific energy consumption curve (SEC) by varying the refining time (3 – 12min) and, thus, also the energy level of the refining. The total energy consumption of the refining was obtained from a watt-hour meter by means of a cumulative pulse counter. The energy consumption value obtained per amount of defibred chips was corrected by a zero load.

The defibration times for the treated spruce sapwood chips were 3 – 12min. The treatments were carried out as described in Example 3 (45°C, 22h). The compression/absorption treatments were carried out using a treated cellulase mixture, as in Example 1, CBH I, and an EG-rich enzyme preparation. The dosages for the treated mixture were 0.63 and 6.3 of protein per g of chips (as dry matter). The dosage for CBH I and the EG-rich enzymes was 5.0mg of protein per g of chips (dry matter). Reference refining operations were carried out on untreated chips and chips that were treated otherwise similarly to the others but without the enzyme (a buffer treatment). After refining, the pulp was removed from the refiner, filtered, homogenized and its dry content was defined, on the basis of which the SEC value could be calculated (kWh/kg).

The results are shown in Table 2.

Table 2.

Treatment	CFS, ml	SEC, kWh/kg
Buffer treatment (pH5)	100ml	4.78
Cellulase mixture, 0.63 mg/g	"	4.15
Cellulase mixture, 6.3 mg/g	"	3.79
CBH I, protein 5mg/g	"	4.94
EG-rich, protein 5mg/g	"	4.14

According to the results, it could be stated that the pre-treatment of chips with the treated cellulase mixture considerably enhanced the beatability, compared with the other cellulase preparations (CBH I and the EG-rich): depending on the enzyme dosage used, energy savings of 10 – 20% were achieved with the cellulase mixture compared with the corresponding buffer treatment.

Example 3

Effect of the enzymatic treatment on the sheet properties of the pulp

The chips were impregnated and treated with a cellulase mixture (a dosage containing 0.63mg of protein per g of chips (dry matter), 45°C, 22h), as described in Example 1. After this, the chips were refined by the blade refiner in accordance with Example 2. Laboratory sheets were prepared from the refined pulps and tested in accordance with SCAN methods. The sheet properties are shown in Table 3.

Table 3.

Treatment	SEC, kWh/kg	CSF, ml	Density, kg/m^3	Tensile index, Nm/g	Tear index, mNm^2/g	Scott Bond, J/m^2	Opacity, %	Brightness, %
Reference	4.61	115	361	38.5	8.24	112	94.5	54.4
Cellulase	4.19	108	375	39.9	7.84	139	93.5	56.0

According to the results, the cellulase treatment, which was applied to the chips, improved the strength properties of the pulp; the tensile strength and the z-strength (Scott Bond) in particular. Also the optical properties were well-preserved.

References

M. Akhtar, M. Attridge, G. Myers, T.K. Kirk & R. Blanchette. Biomechanical pulping of loblolly pine with different strains of the white-rot fungus *Ceriporiopsis subvermispora*. TAPPI J. 75 (1992), 105-109.

Chen H., Hayn M. & Esterbauer H. Purification and characterization of two extracellular β-glucosidases from Trichoderma reesei. Biochim. Biophys. Acta 1121 (1992), 54-60.

Covert, S., Vanden Wymelenberg, A. & Cullen, D., Structure, organisation and transcription of a cellobiohydrolase gene cluster from *Phanerochaete chrysosporium*, Appl. Environ. Microbiol. 58 (1992), 2168-2175.

Eriksson, L.A. & Heitman, J.A. Jr. Enzyme treatment of wood chips for mechanical pulping and the resulting effects on wood and fiberultrasructure. 7 th Int. Conf. Biotechnol. Pulp Pap. Ind., 1998, Volume B, B25-B28.

D.A. Goring. Thermal Softening of Lignin, Hemicellulose and Cellulose. Pulp And Paper Magazine of Canada 64 (1963) 12, T517-27.

Grethlein, H.E. Biotechnology, February 1985, pp.155 – 160.

Ito, S., Shikata, S., Ozaki, K., Kawai, S., Okamoto, K., Inoue, S., Takei, A., Ohta, Y. & Satoh, T., Alkaline cellulase for laudry detergents: production by *Bacillus sp.* KSM-635 and enzymatic properties, Agril. Biol. Chem. 53 (1989), 1275-1281.

K. Jokinen & M. Savolainen. Puun mekaanisen massan käsittely lakkaasilla. PSC Communications 18. Espoo 1991.

Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (1970), 680-685.

G. Leatham, G. Myers & T. Wegner. Biomechanical pulping of aspen chips: energy savings resulting from different fungal treatments. TAPPI J. **73** (1990), 197-200.

Mitsuishi, Y., Nitisinprasert, S., Saloheimo, M., Biese, I., Reinikainen, T., Clayssens, M., Keränen, S., Knowles, J. & Teeri, T. Site-directed mutagenesis of the putative catalysic residues of *Trichoderma.*

Paloheimo, M., Miettinen-Oinonen, A., Torkkeli, T., Nevalainen, H. and Suominen, P. Enzyme production by Trichoderma reesei using the cbh I promoter. Proceedings of the second TRICEL symposium on *Trichoderma reesei* cellulases and other hydrolases, Espoo, Finland, 1993, ed. by P. Suominen & T. Reinikainen, Foundation for Biotechnical and Industrial Fermentation Research & (1993):229-238.

Penttilä, M., Antre, L., Lehtovaara, P., Bailey, M., Teeri, T. & Knowles, J. Effecient secretion of two fungal cellobiohydrolases by Saccharomyces cerevisiae. Gene 63 (1988) 103-112.

J-C Pommier, J-L Fuentes & G. Goma. Using enzymes to improve the process and the product quality in the recycled paper industry. Part 1: the basic laboratory work. TAPPI J. 72 (1989) 6, 187-191.

J-C Pommier, G. Goma, J-L Fuentes, C. Rousser, O. Jokinen, Using enzymes to improve the process and the product quality in the recycled paper industry. Part 2: Industrial applications. TAPPI J. 73 (1990) 12, 197-202.

Rydholm, S. Pulping Processes, Interschience Publishers, London, 1965.

E. Setliff, R. Marton, G. Granzow & K. Eriksson. Biochemical pulping with white-rot fungi. TAPPI J. 73 (1990), 141-147.

Ståhlberg, J. Functional organization of cellulases from *Trichoderma reesei*. PhD Thesis. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science, No. 344, Uppsala, 1991.

Suominen, P. et al., 1993. High frequency one-step gene replacement in Trichoderma reesei. II Effects of deletions of individual cellulase genes. Mol. Gen. Genet. 241:523-530.

Teeri, T., Salovuori, I. & Knowles, J., The molecular cloning of the major cellobiohydrolase gene from *Trichoderma reesei* Bio/Technolgy 1 (1983), 696-699.

Tomme, P., McCrae, S., Wood, T. & Claeyssens, M. Chromatographic separation of cellulolytic enzymes. Methods Enzymol. 160 (1988), 187-193.

van den Hondel, C., Punt, P. & van Gorcom, R. Production of extracellular proteins by the filamentous fungus *Aspergillus*. Antonio van Leeuwenhoek 61 (1992), 153-160.

van Tilbeurgh, H. Bhikhabhai, R. Pettersson, L. and Claeyessens M. (1984) Separation of endo- and exo-type cellulases using a new affinity method. FEBS Lett. 169, 215-218.

Virkola, Nils-Erik (Ed.) Puumassan valmistus. Suomen Paperi-insinöörien yhdistys. Turku 1983.

Zurbriggen, B.Z., Bailey, M.J., Penttilä, M.E., Poutanen, K. and Linko M. (1990) Pilot scale production of a heterologous *Trichoderma reesei* cellulase in *Saccharomyces cerevisiae*. J. Biotechnol. 13, 267-278.